MED64 The most sensitive microelectrode array system for *in vitro* extracellular electrophysiology

MED64 Protocol

Primary Neuronal Cell Culture

ALPHA MED SCIENTIFIC

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1. Introduction

Dissociated neuron cultures offer several distinct advantages for investigating the electrical and chemical communication between neurons. These advantages can be applied to neuronal models of learning and memory, development, aging, disease, and many more. The emergence of the MED64 as the industry leader in micro- electrode array technology has made it a popular platform for studying the electrophysiological properties of neuron cultures.

The MED64's broad acquisition bandwidth combined with superior signal to noise ratio affords impeccable extraction of basic electrophysiological variables. The combination of precise extraction of electrophysiological activity with the ease of culturing cells directly onto the MED Probe (Multi Electrode Dish) makes the MED64 ideal for pharmacological, drug safety screening, and basic scientific applications. The 64 electrodes on the MED Probe have the lowest impedance of any micro-electrode array, making the MED64 ideal for acquiring data from spontaneously spiking neurons or neurons that fire in response to drug application. The MED64 also has high capacitance electrodes, enabling the MED64 to deliver high stimulating current, which is essential for evoked response studies.

The goal of this application note is to describe how to set up experiments with dissociated neuron cultures, acquire relevant data, and extract the data for presentation or publication. This material has been prepared by scientists with expertise in neuroscience and electrophysiology. A complete protocol for plating, culturing, and carrying out experiments on dissociated neuron cultures has been prepared based on the users' experience.

1-1. Acknowledgment

Alpha MED Scientific would like to thank the MED64 users that have shared their knowledge,:

Ikuro Suzuki, PhD - Tohoku Institute of Technology
Aoi Odawara - Tohoku Institute of Technology
Keiichi Shirakawa, PhD- Application Scientist, Alpha MED Scientific
Michael Trujillo, PhD - Senior Application Scientist, Alpha MED Scientific

1-2. Disclaimer

This application note is a summary of information shared by MED64 users and is to be considered marketing material. These methods have been developed, tested, and verified in the course of projects published in peer-reviewed literature. However, Alpha MED Scientific does not guarantee that the information written in this document is correct and is free from all liabilities. Please refer to the scientific literature for further insight on these techniques, as well as the MED64 and Mobius manuals for detailed instructions on use of the MED64 System.

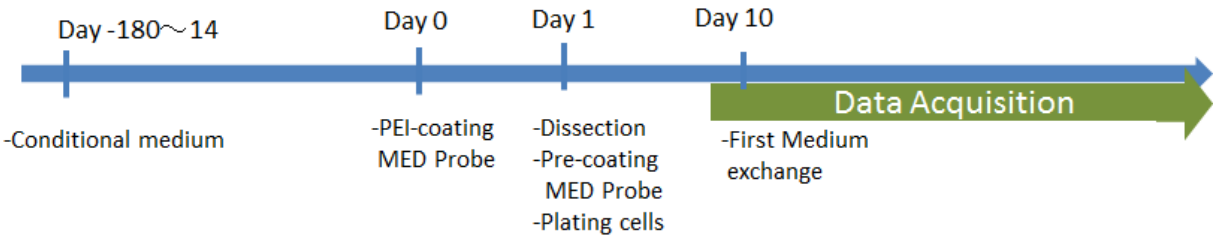
2. Pretreatment of the MED Probes and plating neurons

2-1. Material to be prepared

Items	Supplifer	Cat. No
Biological material		
Rat	Various	
Chemicals		
Polyethyleneimine	Sigma	P3143
Na2B4O7/10H2O	Sigma	S9640
Ethanol (70%)	Various	
Laminin-511 *1	Nippi	iMatrix-511
Poly-D-lysine *2	Sigma	P1149
Ether	Various	
FBS	Various	
HBSS	WAKO	084-08342
Pryruvic acid	Sigma	107360
HEPES	Sigma	H3375
Dnase	Roche	10-104-159-001
Trypsin	15090-046	15090-046
Neurobasal Medium	Life Technologies	21103-049
B27 supplement	Life Technologies	17504044
L-glutamine	Life Technologies	21051-024
Penicillin-Steptomtsin	Life Technologies	15140-148
Equipment		
MED Probe	Alpha MED Scientific	MED-P515A
Scissors	Various	
Forceps	Various	
Pipet	Various	
Petri dish	Various	
Cornical tubes	Various	
6-well culture plate	Cell culture type is recommended	
Kimwipe	Various	
Waterbath	Various	
Clean bench	Various	
Centrifuge	Various	
Microscope	Various	

Either of *1 or 2 will be used for pre-coating the MED Probe.

2-2. Workflow for preparation



2-3. Pretreatment of the MED Probe

CAUTION:

Avoid contact with the electrodes in all of following procedures as they are extremely fragile.

Pretreatment of the MED Probe is the most critical step to culture neurons onto the electrodes. The surface of a new MED Probe is hydrophobic. Therefore, hydrophilization of the MED Probe is necessary to enhance the adhesion of neurons to the electrodes. If cells do not adhere to the electrodes, you will not be able to record from them. It should be noted that some coating agents can affect neural activity, viability, degree of neurite outgrowth, extent of migration, and longevity. Improper coating techniques can cause large-scale clumping and/or the death of neurons even if all other cell culture steps are performed properly. The following section contains recommendations for appropriate pretreatment of the MED Probes including 2 different coating methods.

Sterilization

1. Rinse a new MED Probe with sterilized distilled water (SDW) at least three times. Rinse it with 70% ethanol several times (or immerse it in 70% ethanol for 15 minute), and then let it dry naturally on a clean bench. Higher-grade ethanol is recommended to avoid deposits of organic substances onto the MED Probe after drying.

2. Rinse the MED Probe with sterilized distilled water (SDW) at least three times, and then let it dry under ultraviolet irradiation for 15-30 min. Store and handle the MED Probe in a sterilized 90 mm petri dish.

Note:

Above steps are not always required, especially when reusing a MED Probe.

Pre-coating with PEI

The surface for the new MED Probe is hydrophobic. Thus, pre-coating with PEI is important for enhancing the hydrophilicity of the MED Probe's surface and preventing clumping of cells during their growth. While Pre-treatment with PEI is not typically required when re-using MED Probes, some MED Probes may need to be pre-treated again after several uses.

Note:

Pre-coating with PEI needs to be made on the day before plating cells.

1. Treat the surface of a MED Probe with 0.005%* Polyethyleneimine (PEI) in 25 mM borate buffer (pH 8.4) for one hour at room temperature. (Make sure the electrodes are covered by the PEI.)
   *If the insulating film of the MED Probe is acrylic, suitable concentration is 0.05%.
2. Aspirate the PEI and rinse the MED Probe with DSW 4 times.
3. Dry the MED Probe on a clean bench overnight.

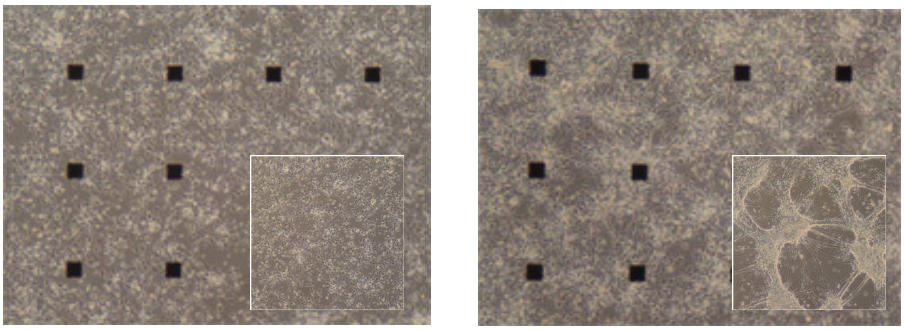


Figure 1. Rat hippocampal neurons in 5 days after plating with PEI pre-treatment (left) and without PEI pre-treatment (right). Cells grew evenly on the MED Probe pre-treated with PEI (left).

Recipe -PEI solution-

<u>25 mM borate buffer</u>

Dissolve 25 mM Na2B4O7/10H2O (MW: 381.4, Sigma: S9640) in distilled water and adjust the pH to 8.4 with HCl. (500 ml borate buffer solution)

1) 4.768g Na2B4O7/10H2O in 450 ml distilled water.

2) Adjust pH to 8.4 with HCl.

3) Add distilled water to 500 ml.

<u>0.005% or 0.05 PEI (polyethyleneimine) solution</u>

Since a 50% PEI solution (Sigma: P3143) is so sticky, it is recommended to prepare a 1% stock solution first (1 ml 50% PEI to 49 mls 25mM borate buffer). This 1% stock solutions is then diluted to (0.005% or 0.05% PEI) for final use.

- The PEI solution can be stored in refrigerator up to 1 month.

Coating method #1: Coating with Laminin-511

Note:

Coating needs to be made on the day of plating cells.

1. Rinse the MED Probe with PBS and fill the MED Probe with 1 ml of Laminin-511 (2 μg/ml). Store the MED Probe in a sterilized petri dish and incubate it for up to 1 hour.

    - Alternatively, the Laminin-coated Probe can be left in room temperature for 3 hours or in a refrigerator (4°C) for overnight.

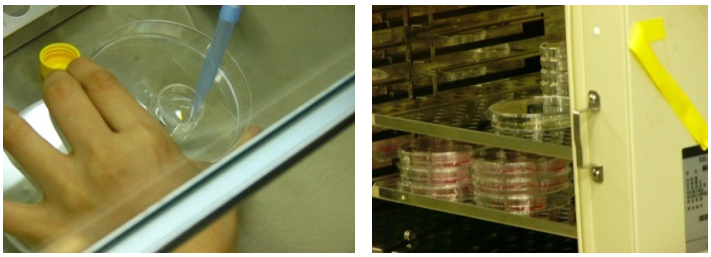


Figure 2. Rinse the MED Probe with PBS (left). Store the MED Probe filled with Laminin-511 in a sterilized dish and incubate it for up to 1 hr (right).

2. Move the MED Probe into a clean bench. Aspirate the Laminin-511 from the MED Probe, If there are bubbles on the electrodes, carefully remove them using a pipette. (Do this on a clean bench.)

    Note:

    Bubbles on the electrodes will block cells grown onto electrodes. Make sure to remove all bubbles.

3. Plate neurons as soon as possible before the electrodes are dried (described in the next section).

CAUTION:

Be careful NOT to touch the electrodes.

Coating method #2: Coating with Poly-D-lysine

Note:

Coating needs to be made on the day of plating cells.

1. Rinse the MED Probe with 50 mg/ml poly-D-lysine solution (M.W. is more than 70000) once, and fill the MED Probe with 1 ml of poly-D-lysine. Store the MED Probe in a sterilized petri dish and incubate it for up to 1 hour.
2. Move the MED Probe into a clean bench. Aspirate poly-D-lysine solution and let it dry naturally.

   Note:

   The electrode surface should not repel water and should maintain moisture after the completion of this process.

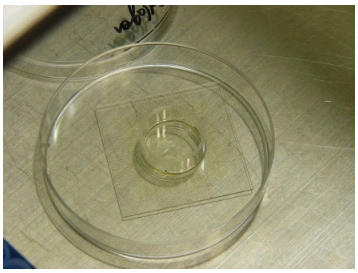


Figure 2. The MED Probe after completion of the coating. The electrodes should maintain moisture.

3. Rinse the MED Probe with SDW at least 3 times. Fill the MED Probe with maintenance medium, and incubate the MED Probe at 37°C for 1 hour.
4. Take the MED Probe from the incubator. If there are bubbles on the electrodes, carefully remove them using a pipette. (Do this on a clean bench.)

   Note:

   Bubbles on the electrodes will block cells grown onto electrodes. Make sure to remove all bubbles.

5. Plate neurons as soon as possible (described in the next section).

CAUTION:

Be careful NOT to touch the electrodes.

2-4. Plating and culturing rat hippocampal neurons

Preparation

1. Fill four 90 mm petri dish (P#1-4) with 20 ml of treatment medium each. (See Table. 1. for composition of the treatment medium.)

Ingredient	Volume /Concentration
HBSS	2L
Pryruvic acid	1 mM
HEPES	10 mM

*Adjust pH to 7.2 with NaOH solution

Table 1. The composition of the treatment medium (for 2L).

2. Fill six conical tubes (C#1-6) with medium listed in the Table 2-3. Warm up conical tube C#2 with a water bath at 37 C. Chill the other conical tubes (#1, 3-6).

Conical tube	Medium	Volume
C#1	treatment medium	15 ml
C#2	0.1 mg/ml DNase (100 μl), 0.5 mg/ml trypsin (100 μl), HBSS	5 ml
C#3	0.1 mg/ml DNase (50 μl), 10%FBS (500 μl), treatment medium	5 ml
C#4	0.1 mg/ml DNase (35 μl), treatment medium	5 ml
C#5	0.1 mg/ml DNase (20 μl), treatment medium	5 ml
C#6	modified Neurobasal medium	5 ml

Table 2. Medium in the 6 conical tubes.

Ingredient	Volume / Concentration
Neurobasal Medium (Life Technologies #21003-049)	500 ml
B27 supplement (Life Technologies #17504044)	10 ml
L-glutamine *	0.074 mg/ml
Penicillin-Streotomycin (Life Technologies #15140-148)	5 ml

* Dissolve 40.7 mg of L-glutamin with 22 ml of Neurobasal medium, and filter it. Use 20 ml.

Table 3. The composition of modified Neurobasal medium (513 ml).

Dissection and plating cells onto the MED Probe

Note: *Refer to Ref.1. for further information.*

1. Anesthetize a pregnant rat with ether. Dissect both carotid arteries and incise the abdomen.
2. Isolate uterus containing a litter of 18-day-old fetal rats and transfer it into a sterilized petri dish.
3. Isolate fetal rats from the uterus and wash them in petri dish #1 (P#1) containing the treatment medium. Then transfer them into petri dish #2 (P#2).
4. Place the fetal rats on a KimWipe. Isolate brain tissue containing cerebellum and transfer it into petri dish #3 (P#3).
5. Isolate both cerebral hemispheres from the brain tissue in the P#3, and transfer them into petri dish #4 (P#4).
6. Isolate both hippocampi in P#4 chilled with flaked ice under a microscope, and transfer them into conical tube #1 (C#1) chilled with flaked ice.

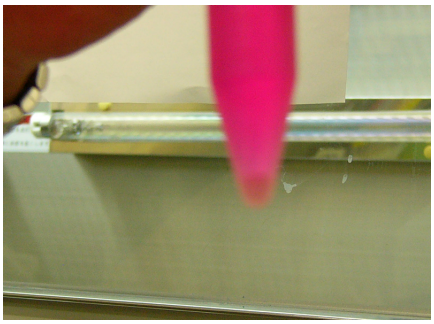


Figure 4. Isolating hippocampi with flaked ice under microscope (left). The hippocampal transferred into C#1 (right).

** Anatomical procedure (1-5) with photos is available. Contact support@med64.com.*

7. Wait for the hippocampi settled in the bottom of conical vial #1 (C#1), and then aspirate "the supernatant of the medium". Add the medium contained in conical vial #2 (C#2) to (C#1).
8. Incubate C#1 for 15 min. Take it out from the incubator every 5 minutes, shake it gently, and return it to the incubator. (This process will work to enhance mixing and avoid damaging cells in later processes and pipetting.)

Figure 5. Add solution to C#1 (left) and incubate it for 15 min. Take it out to shake gently every 5 minutes (right).

9. Centrifuge C#1 with 1000 rpm at 4°C for 5 min.

10. Aspirate the supernatant and add the medium contained in conical tube #3 (C#3) to C#1. Pipette C#1 gently ten times, then centrifuge it with 1000 rpm at 4°C for 5 min.

11. Aspirate the supernatant and add the medium contained in conical tube #4 to C#1. Pipette C#1 gently ten times, then centrifuge it with 1000 rpm at 4°C for 5 min.

12. Aspirate the supernatant and add the medium contained in conical tube #5 (C#5) to C#1. Pipette C#1 gently ten times, then centrifuge it with 1000 rpm for 5 min at 4°C.

13. Aspirate supernatant and add solution contained in conical tube #6 to C#1. Pipette C#1 gently twenty times to suspend completely.

Figure 6. Centrifuging

14. Filter the cell suspension with 40 μm cell strainer.

15. Mix your conditional medium with modified Neurobasal Medium (in C#6) with the ratio of 1:3. Filter it with 0.22 μm filter and then pour it into the coated MED Probe.

16. Plate the 5 x 10^5 cells suspension over the microelectrodes grids as soon as possible after the 15th step. (e.g. 100 μl for 5 x 10^6 cells/ml cells suspension). If there are air bubbles around electrodes, pipette them gently to remove.

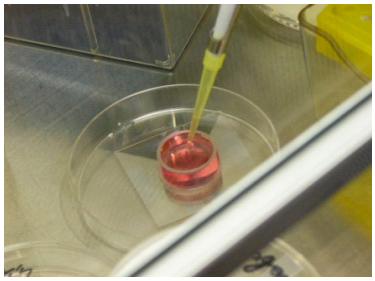
    Note:

    The MED Probe has 4 reference electrodes surrounding 64 recording electrodes. These need to be free from cells. On the other hand, these 4 reference electrodes must be covered completely by medium because the field potentials around recording electrodes are compared to the reference electrodes with the MED64 System.

17. Exchange half of the mixed-medium (10%:conditional medium / 90%:modified Neurobasal medium) with fresh medium 10 days after plating. Exchange half the medium once every 1-2 weeks after this first change.

    Note:

    It typically takes around 24 hours after the medium change to be able to record good and stable signals.



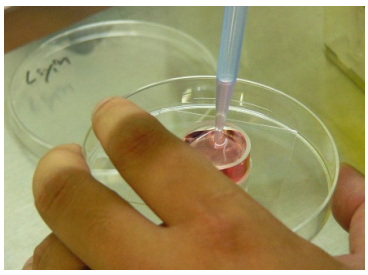


Figure 7. Exchanging the medium.

18. It typically takes 7-10 days after plating for neurons to form networks dense enough to record field potentials.

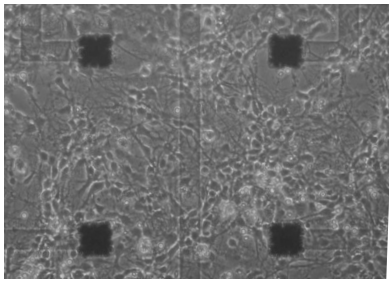


Figure 8. Neurons cultured for 7 days on a MED Probe.

Conditional medium

Culturing with conditional medium is recommended to let neurons grow rapidly and keep them in good conditions. This will enhance the signal to noise ratio when recording. It is essential to allow glial cells to grow moderately. An important method to achieve this is setting the concentration of fetal bovine serum (FBS) to 1% in the culture.

Here is a recipe for conditional medium:

1. Plate glial cells and neurons with 10% FBS and modified Neurobasal medium in a 6-well culture plate.
2. Culture them for 7-10 days until they are 100% confluent. (Medium turning to yellow is a sign for confluency.)
3. Remove the 2/3-3/4 of medium and add the same volume of FBS (10%). Culture for another week.
4. Remove the supernatant (half of entire medium), and add the same volume of FBS (10%). Filter the supernatant with 0.22 μm filter and store it at -20°C. (Don't mix medium from another day.)
5. Thaw it in a water bath at 37°C before use.

Note:
The same 6-well plate (containing glial cells) can be used for up to 3 months if medium is exchanged once per week.

2-5. Cleaning the used MED Probes

The MED Probe's electrical characteristics are best during the first use. High quality signals can be recorded and effective stimulation is possible with the MED64 System's MED Probes thanks to the electrode's characteristics, the lowest impedance in a commercially available microelectrode array. The electrode's impedance will increase with repeated use of the MED Probes due to damage in handling and/or residual cellular debris and coating materials. However, the MED Probes can be re-used if they are handled and cleaned very carefully. The following are protocols MED64 users recommend:

CAUTION:

ALWAYS avoid contact with the surface of the MED Probe to preserve the microelectrodes and insulation layer.

Trypsin method

1. Fill a used MED Probe with 0.25% Trypsin, and incubate at 37°C for 1 hour.
2. Rinse the MED Probe with PBS 3 times.
3. Rinse the MED Probe with sterilized distilled water (SDW) at least 3 times.
4. Fill the MED Probe with SDW, and store the MED Probe in a 90 mm petri dish.
5. Store the petri dish in refrigerator.

Bleaching method

CAUTION:

This method is available only for Platinum-Black electrodes-MED Probes. Do NOT use this method for CNT electrodes-MED Probes.

1. Carefully pour or pipette 1 ml of Bleach (Clorox®) into the MED Probe and leave it for about 15-30 seconds.
2. Aspirate and repeat step 1 three times. If the MED Probe is not clean after this, apply 2 more rinses and a longer exposure. (usually 1-2 minutes is enough, but as long as 15 minutes is acceptable.)

   CAUTION:

   Avoid exposing the MED Probe to Bleach over 20 minutes.
3. Rinse the chamber 5 times with double distilled water (DDW).
4. Allow it to dry or aspirate in a sterile hood.
5. Store the MED Probe (according to instructions) in a beaker with DDW in a cool dark area. (Please refer to the MED Probe product manual.)

3. Data acquisition

Cultured neurons are spontaneously active within 7-10 days after seeding, but initial activity can vary depending on culturing technique. Once the neurons begin firing spontaneously, activity can be recorded using the MED64. The recommended environment for recording from dissociated neurons is in the following section.

3-1. Recommended experimental environment

37°C is the typical recording temperature recommended for recording from dissociated neuron cultures However, the set temperature can vary depending on samples and nature of your experiments. To maintain the desired temperature, one of two methods are recommended.

1) Use of CO_2 incubator

Place the MED Connector (MED-C03) inside a CO_2 incubator. Please note that incubators can introduce noise. Please refer to page 29-36 in the MED64 Handbook (vol1) to mitigate noise introduced by an incubator. Particularly, recordings can be compromised by noise introduced by the rapid temperature increases that occur as incubators power on. Wait until the desired temperature is stable before beginning acquisition. (It could take several hours depending on the incubator.)

Figure 9. Experiment with the MED Connector inside a CO2 incubator.

2) Use of the MED Heated Connector

The MED Heated Connector (MED-CP02H) heats the MED Probe chamber from the bottom. Below are recommendations for using the MED Heated Connector.

1. Do NOT place the MED Heated Connector in an environment where temperature changes frequently, for example in the proximity of an air conditioner or heater.

2. Cover the MED Probe to prevent ambient temperature fluctuation during acquisition.

3. It can take some time to reach the set-temperature. Power on the ThermoClamp^{TM}-1 (controller for the MED Heated Connector) connected to the MED Heated Connector at least 30 minutes before starting acquisition.

4. If/When the set-temperature is changed, wait for the temperature reported by the ThermoClamp^{TM}-1 controller to stabilize at the new temperature. It could take 5-30 minutes or more but is typically very fast.

3-2. Data acquisition

The MED Probe has 64 recording electrodes as well as 4 reference electrodes. The differences between the field potential acquired at the recording electrodes and the potential at the reference electrodes are measured by the MED64 System. Acquired signals are sent to the MED64 Head Amplifier through the MED Connector/MED Heated Connector. The raw signals are amplified by x10 by the Head Amplifier, and then amplified further and digitized with the MED64 Main Amplifier. We highly recommend reading the "Product manual" for each component, as well as the "MED64 Handbook", and "Mobius Tutorial" before using the MED64 System.

Required MED64 System Components

1) MED Probe
2) MED Connector (MED-C03) / MED Heated Connector (MED-CP02H) *1
3) MED64 Head Amplifier (MED-A64HE1S)
4) MED64 Main Amplifier (MED-A64MD1)
5) Acquisition PC
6) Mobius software (Mobius Spike Sorter or Mobius Spike Sorter with stim package)

*1. MED Heated Connector requires the ThemoClamp^{TM}-1 controller.

1. Sanitize the MED (Heated) Connector by cleaning with kim-wipe soaked with ethanol. (Don't clean the contact pins in the Connector).

2. Mount the MED Probe containing the dissociated cells onto the MED (Heated) Connector.

CAUTION:

Clean the terminals on the outer portion of the MED Probe with a Kimwipe soaked in ethanol before mounting the MED Probe. Salt sediments can damage the contact pins on the MED (Heated) Connector.

3. Cover the MED Probe with a 35 mm petri dish or Perfusion Cap.

Available Mobius workflow templates

Data can be acquired immediately using the available Mobius workflow templates enumerated below (please refer to page 43, Chapter 3: Spike Sorter on the Mobius Tutorial):

1. Spontaneous_recording (Recording WITHOUT any real-time analysis).
2. Spike_recording (Recording with extracting spikes and their frequency analysis).
3. Spike_recording_filter (2 + filter).
4. Spike_recording_cluster (Recording with extracting, clustering spikes, and their frequency analysis).
5. Spike_recording_filter_cluster (4+filter).

Recommended acquisition settings

The following parameters are recommended to perform data acquisition and analysis of neuronal spikes:

Input Range (Maximum input signal level):	2.9 mV
Low cut freq (High pass filter) :	100 Hz
High cut freq (Low pass filter):	10000 Hz

Acquisition bandwidth can be narrowed to further decrease the baseline noise level.

4. Data analysis

The MED64 can easily record neuronal spikes from active neurons. The Mobius Spike Sorter package is available to extract and analyze neuronal spikes. Analyses as well as raw data can be exported for processing using other software packages.

4-1. Workflow templates available for analyzing neuronal spikes

The Mobius Spike Sorter package has 4 built-in analysis workflow templates available for analyzing neuronal spikes.

"Spike_frequency_analysis", "Spike_frequency_analysis_filter"

These workflow templates allow you to:

- Set thresholds.
- Extract spikes that go over the pre-determined thresholds.
- Counts spike frequencies for the extracted spikes.
- Graph the spike frequency chart.
- Export raw data
- Export waveform of extracted spikes and their time stamps.
- Export the spike frequency chart.

For the *"Spike_frequency_analysis_filter"* workflow template, all above analyses are performed on filtered data.

"Spike_sorting", "Spike_sorting_filter"

These workflow templates allow you to perform same analysis as above. However, the extracted spikes are clustered based on their waveform similarities. The spike frequency is computed independently for each cluster.

For the *"Spike_sorting_filter"* workflow template, all analyses are performed on filtered data.

4-2. Setting thresholds

All spike sorter workflow templates build in the [Extract Spikes Advanced] module, where thresholds are set by:

1. Typing numbers in the chart. (Top-left in the Figure 10)
2. Moving bars in the single channel display. (Top middle in the Figure 10)
3. Setting thresholds as SD (Standard Deviation) percentage. (Top right in the Figure 10)
4. Moving bars in the 64ch display. (Bottom in the Figure 10)

Spikes that go over the thresholds are extracted and their frequencies analyzed.

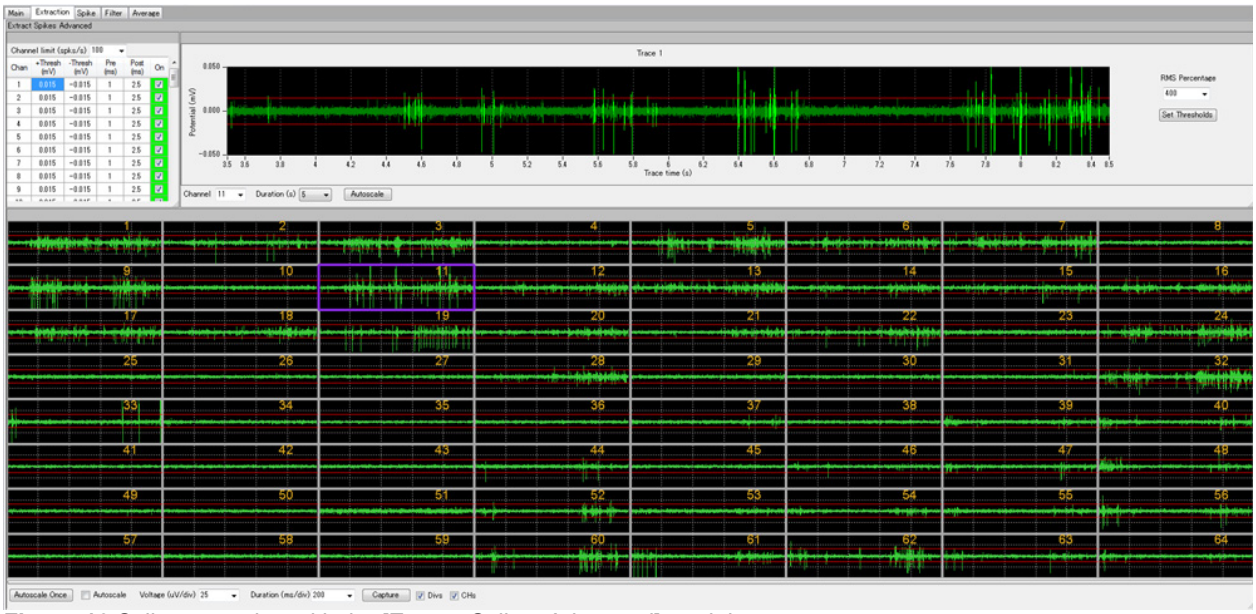


Figure 10 Spike extraction with the [Extract Spikes Advanced] module.

4-3. Spike frequency analysis

Mobius Spike Sorter's built-in "Spike_frequency_analysis_(filter)" workflow templates can extract neuronal spikes and analyze their spike frequencies during recording and/or post-acquisition. Time course of spike frequencies are computed and graphed. Please refer to page 63 in the Mobius Tutorial for detailed instructions.

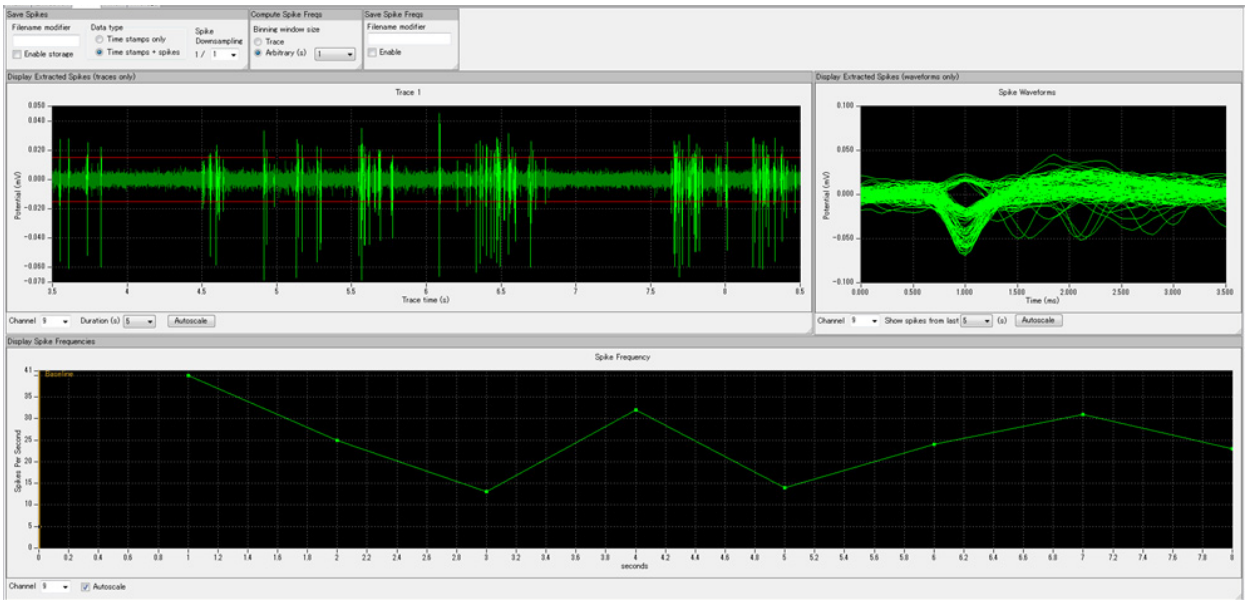


Figure 11. Spike frequency analysis using the *"Spike_frequency_analysis_filter"* workflow template. Spike frequencies for all extracted spikes are computed and graphed at each electrode.

4-4. Spike Sorting

The *"Spike_sorting_(filter)"* workflow templates extract neuronal spikes, sort them based on similarity of waveforms, and analyze their spike frequencies.

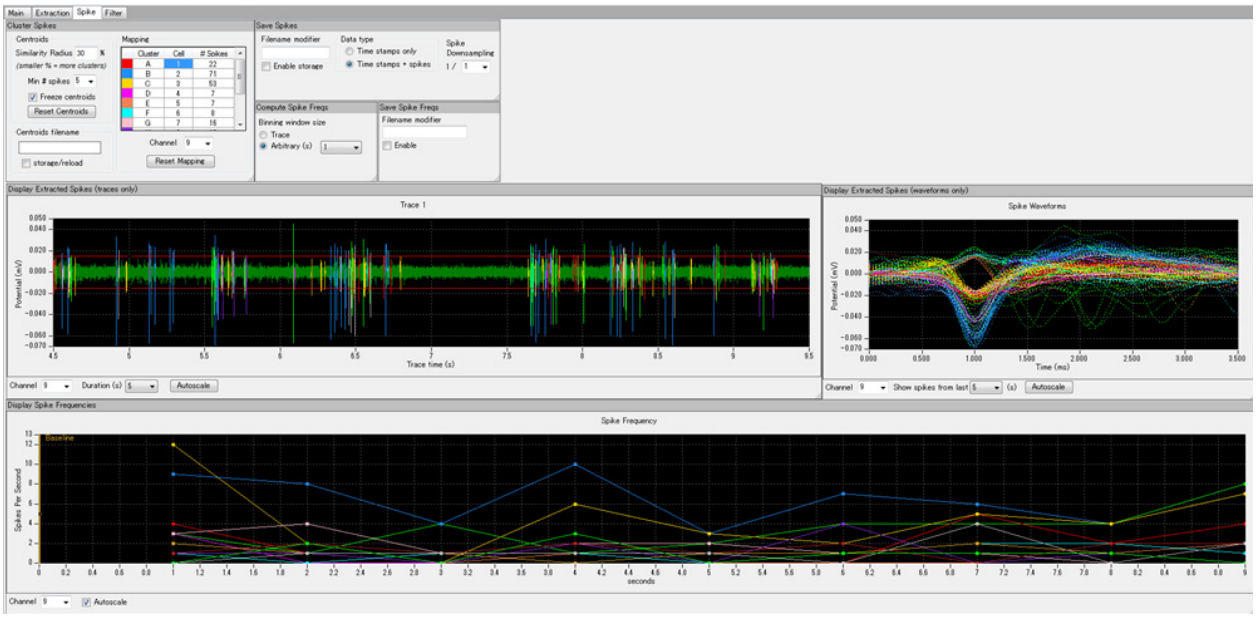


Figure 12. Spike sorting.

4-5. Exporting Data

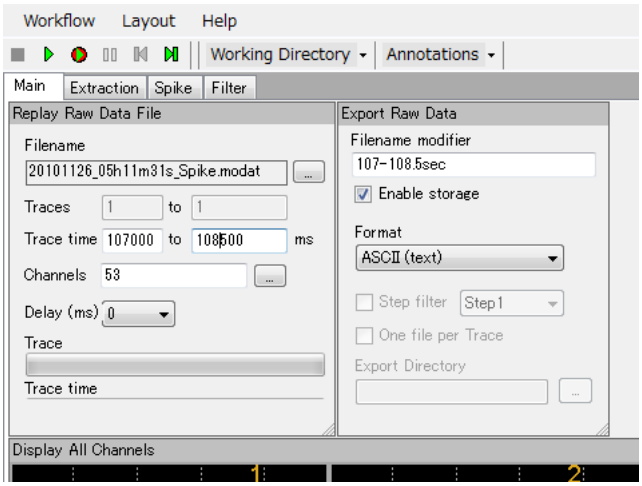
Mobius can export data in user friendly formats for analysis with other software.

Data to be exported	Module	File type
Raw data	Export Raw Data	Binary / ASCII
Time stamp for extracted spikes	Save Spikes	ASCII
Waveform for extracted spikes	Save Spikes	ASCII
Values for spike frequencies	Save Spike Freqs	ASCII
Waveform for centroids	Cluster Spikes	ASCII

Data is exported as ASCII (or binary for Raw data) when:

1) The check-box for the module is checked, and
2) Data is replayed with the Green/Red button.

Channels for exporting are selected via the channel selector in the [Replay Raw Data File]. Traces and times to be exported are also selected at the trace/time counter in the [Replay Raw Data File].



File Format Version	20071201
Session Start Time	2010/11/26 20:11:31 +09
time_ms	ch53_mV
107000	0
107000.05	-0.006836146
107000.1	-0.001953185
107000.15	-0.004394665
107000.2	-0.001953185
107000.25	0.000976592
107000.3	-0.005371258
107000.35	-0.008301035
107000.4	-0.002929777
107000.45	-0.002929777
107000.5	-0.004394665
107000.55	-0.007324442
107000.6	-0.000976592
107000.65	-0.001464888
107000.7	-0.007324442
107000.75	-0.003906369
107000.8	-0.002441481

Figure 13. [Left] Setting for exporting raw data with the Mobius (left). 107000-108500 ms of ch 53 are selected in the [Replay Raw Data File] module while ASCII is selected and Enable storage is checked in the [Export Raw Data] module. Data will be exported by clicking the green/red button.
[**Top-right]** A part of raw data exported by ASCII and displayed in Excel.
[**Bottom-right]** Waveform created by using Excel.

5. References

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